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Richness and Diversity of Bacterioplankton Species along an Estuarine Gradient in Moreton Bay, Australia

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Bacterioplankton community diversity was investigated in the subtropical Brisbane River-Moreton Bay estuary, Australia (27°25′S, 153°5′E). Bacterial communities were studied using automated rRNA intergenic spacer analysis (ARISA), which amplifies 16S-23S ribosomal DNA internally transcribed spacer regions from mixed-community DNA and detects the separated products on a fragment analyzer. Samples were collected from eight sites throughout the estuary and east to the East Australian Current (Coral Sea). Bacterioplankton communities had the highest operational taxonomic unit (OTU) richness, as measured by ARISA at eastern bay stations (S [total richness] = 84 to 85 OTU) and the lowest richness in the Coral Sea (S = 39 to 59 OTU). Richness correlated positively with bacterial abundance; however, there were no strong correlations between diversity and salinity, NO_3^- and PO_4^{3-} concentrations, or chlorophyll *a* concentration. Bacterioplankton communities at the riverine stations were different from communities in the bay or Coral Sea. The main differences in OTU richness between stations were in taxa that each represented 0.1% (the detection limit) to 0.5% of the total amplified DNA, i.e., the "tail" of the distribution. We found that some bacterioplankton taxa are specific to distinct environments while others have a ubiquitous distribution from river to sea. Bacterioplankton richness and diversity patterns in the estuary are potentially a consequence of greater niche availability, mixing of local and adjacent environment communities, or intermediate disturbance. Furthermore, these results contrast with previous reports of spatially homogeneous bacterioplankton communities in other coastal waters.

Estuaries are among the most productive marine ecosystems on earth; however, they are also subject to the greatest pressures from coastal human populations. On the east coast of Australia, several estuaries have become eutrophied as a consequence of human activity, and consequently, they have strong gradients in productivity and nutrient availability (4). In these, as in other estuaries and adjacent waters, bacteria (including cyanobacteria) are abundant and productive components of plankton, with typical abundances of 10^5 to 10^7 cells ml^{-1}, and typically represent 70% of the carbon biomass in upper surface waters (8). Despite their biogeochemical importance, little is known of the composition, and consequently, of the patterns of diversity, of bacterioplankton communities in most marine environments (14). The study of bacterioplankton communities has traditionally been limited by dependence upon culture-based techniques (13), but only a small fraction of bacterioplankton species are culturable (19). Thus, modern study of these communities has focused upon molecular techniques that circumvent the limitations of cultivation (11).

Advances in DNA technology have allowed detailed investigation into the diversity and species richness of bacterioplankton communities. Early studies of the molecular diversity of bacteria in the ocean focused upon the cloning and sequencing of amplified conserved genes, such as 16S rRNA (13). This method allows high phylogenetic resolution of bacterioplankton communities, because identity is based upon sequence information. However, recent study has demonstrated that a very large number of clones need to be processed to accurately estimate the absolute species richness of bacterioplankton communities by this approach (18), making it both time-consuming and expensive. Thus, while the approach is useful for describing bacterioplankton communities, it is often impractical to use clone libraries quantitatively, especially for relatively rare components that require especially large libraries.

In recent years, whole-community fingerprinting approaches have been used to study complex bacterial communities and to estimate the diversity and relative representation of individual bacterial taxonomic units within the total detectable bacterial communities. Three common fingerprinting methods are terminal restriction fragment length polymorphism (TRFLP) of universally conserved genes (2), denaturing gradient gel electrophoresis (DGGE) (35), and automated rRNA intergenic spacer analysis (ARISA) (3, 9). In TRFLP, 16S rRNA is amplified by PCR with a 5′ fluorescent primer, and amplicons are digested into fragments using restriction enzymes, resulting in terminal restriction fragments of distinctive lengths (2). DGGE relies on melting-point variations in variable portions of target molecules, often 16S rRNA. ARISA amplifies the region between 16S and 23S rRNAs using a fluorescent primer; this portion of the operon is highly variable in length (from 150 to 1,200 bp), and therefore, digestion of amplicons is not necessary (3). We chose to use ARISA in this study for a few reasons. TRFLP provides less phylogenetic resolution because it relies upon only a few sequence heterogeneities in a generally conserved molecule. DGGE, on the other hand, offers high phylogenetic resolution but has less sensitivity than ARISA or TRFLP for minor taxa; ARISA and TRFLP typically use a

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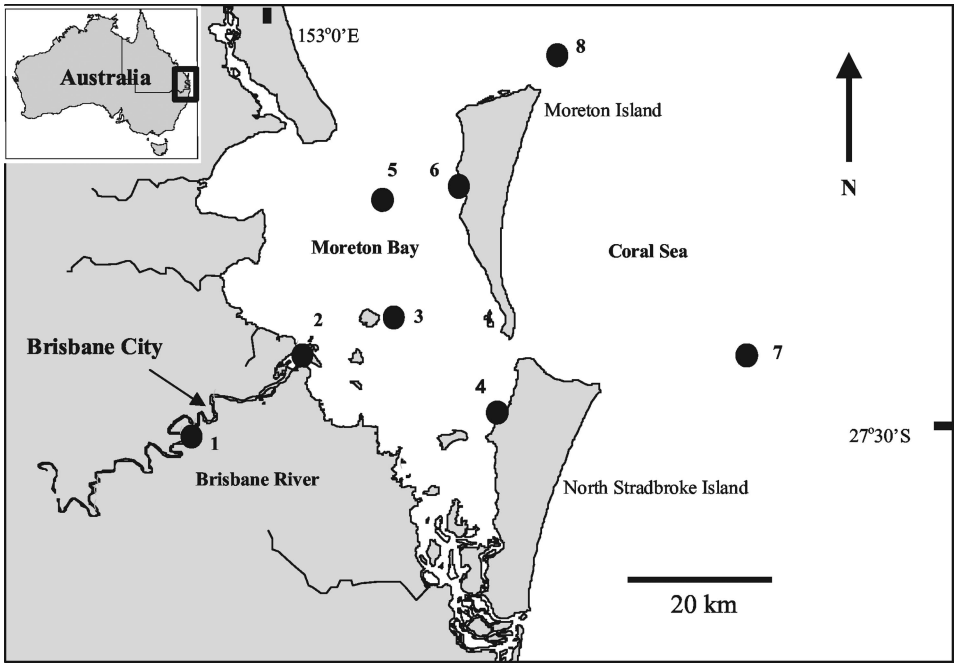

FIG. 1. Map of Brisbane River-Moreton Bay estuary showing sampling sites.

laser detection system that can detect bands and peaks containing <0.1% of the total loaded DNA, while the images or scans of DGGE gels often require >0.5%. Also, DGGE relies on gel band positions that cannot easily be converted into standard data points (unlike digital fragment lengths in ARISA or TRFLP); therefore, it is difficult to standardize or to compare between laboratories. Additionally, DGGE gels are typically shorter than ARISA or TRFLP sequencing gels and therefore permit fewer possible operational taxonomic units (OTU). With respect to speed and cost of analysis, these approaches are all advantageous compared to clone library approaches, since mixed DNA from an entire bacterial community is amplified and visualized within a single assay. However, these methods are not as sensitive to taxonomic differences as clone library approaches, and it has been argued that they distinguish near the genus (TRFLP) and species (ARISA) levels (2, 9).

Sampling the richness and diversity of microbial communities has received considerable recent attention (for a review, see reference 18). Analytical methods of sampling bacterial diversity, such as clone libraries and whole-community fingerprinting, have been criticized, since inherent biases are introduced by using PCR to amplify community DNA. Like all ecological techniques, fingerprinting is not perfect, since inevitably components of communities are not accounted for and some components may be overestimated. Biases can be reduced by limiting the number of PCR cycles, which prevents overamplification of minor peaks (9). Maintaining high stringency can also prevent the formation of spurious products which could be misinterpreted as phylotypes during fingerprinting analysis (9). Another possible complication that arises from the use of ARISA for analyzing bacterioplankton communities is that some species (especially fast-growing ones which have multiple rRNA operons [21]) have more than one internally transcribed spacer (ITS) length, since it is not as conserved as 16S rRNA sequence and there is heterogeneity in operon copy numbers within cells (21). However, within slow-growing bacterial communities, the copy number of the rRNA operon is low, and thus, heterogeneity is less likely to affect fingerprinting analysis in slow-growing marine bacterioplankton communities (2, 3, 21, 27). Fingerprinting is reproducible and suitable for displaying clear differences between communities (9). Furthermore, since the information generated on bacterial communities by fingerprinting can indicate the rank abundance of microbial communities, it indicates the coverage of each microbial community (18). It can be argued that fingerprinting is the most cost-effective alternative for comparing multiple bacterial communities.

The aim of this study was to determine patterns of bacterioplankton diversity along a subtropical estuarine gradient of Moreton Bay, Australia. Such an estuary provides an ideal model system in which a sharp gradient of environmental conditions, yet without physical barriers, can be studied in a short time. Our results demonstrate that bacterioplankton communities vary significantly along the estuarine gradient and that diversity as measured by ARISA may be related to habitat and resource availability. We also demonstrate that ARISA, with its high phylogenetic resolution, is a useful technique for characterizing estuarine and marine bacterioplankton communities.

(This work was conducted in partial fulfillment of the requirements for a Ph.D. by I.H.)

MATERIALS AND METHODS

Sampling locations. Samples were collected in the Brisbane River and Moreton Bay estuary (27°25′S, 153°5′E), a semienclosed bay on the East coast of Australia (Fig. 1). The bay is characterized by a strong east-west trophic gradient as described elsewhere (15). The Brisbane River (sites 1 and 2) is eutrophic and

has a high suspended-matter load (average, 46 mg liter^{-1}), which has been postulated to limit rates of primary production (26). The bay portion (sites 3, 4, 5, and 6), on the other hand, is characterized by oligotrophic conditions and low suspended-matter concentrations characteristic of oceanic conditions (7). Site 7 is in the East Australia Current, which is a warm-water current running southward from the Coral Sea. Site 8 (Flinder's Reef) is near a small and highly diverse coral reef (7). Moreton Bay is a dynamic estuary with a longest seawater residence time of ~70 days within the Brisbane River; however, within the bay itself, residence times range from 6 to 48 days (7). The bay portion of the estuary is tidally and wind mixed and has various habitat types and inputs; thus, bacterial communities experience temporally fluctuating conditions of nutrient availability, illumination, and energy compared to both open-ocean and riverine stations (7). The total water depths at all stations except 7 and 8 were <10 m. At station 7, the water depth was >200 m, and at station 8 it was ~12 m.

Sample collection. Sampling was conducted over 3 days in December 1999 and January 2000. Samples were taken on board the R/V Sea Wanderer II and R/V Porpita (University of Queensland) directly from surface water using an acid-washed and seawater-rinsed bucket. Seawater (4 liters) was initially collected in 4-liter low-density polyethylene water carriers and placed into a cooler to maintain the temperature (±2°C) until samples were processed in the laboratory at the Moreton Bay Research Station (Stradbroke Island, Australia) or at the University of Queensland (Brisbane, Australia) ~6 h after collection.

Samples were prefiltered through 47-mm-diameter Whatman GF/F filters (nominal pore size, 0.7 μm) to remove large particles and protists and then filtered through a 0.2-μm-pore-size Durapore (Millipore) filter. The filters were then placed into sterile plastic Whirlpak bags (Nasco Inc.) and stored at −80°C until analysis was done at the University of Southern California (Los Angeles). Salinity was measured using a HORIBA UD-10 probe, nutrient (NO_3^- and PO_4^{3-}) concentrations were analyzed by standard colorimetric methods (28), chlorophyll *a* was measured by acetone extraction and fluorometry (28), and bacterial abundance was determined using SYBR Green I staining and epifluorescence microscopy (25) as part of a concurrent study (15).

DNA extraction. DNA was extracted from the Durapore filters using protocols described previously (10). After the Durapore filter was placed in a microcentrifuge tube, 500 μl of STE (100 mM NaCl, 10 mM Tris, 1 mM EDTA)–10% sodium dodecyl sulfate (9:1) was added and the tubes were placed in a boiling water bath for 2 min to lyse bacterial cells. After centrifugation at 3,000 × *g* for 5 min, the supernatant was transferred to a new microcentrifuge tube, and DNA was precipitated at −20°C overnight after the addition of 150 μl of 10.5 M NH_4OAc and 1 ml of 100% ethanol. After precipitation, samples were spun at 12,000 × *g* for 30 min at 4°C to pellet the DNA. The pellets were air dried and resuspended in 200 μl of Tris-EDTA (pH 7.8). The resuspended DNA was then extracted sequentially with 200 μl of phenol, 200 μl of phenol-chloroform (10:1), and then 200 μl of chloroform-isoamyl alcohol (24:1). Samples were precipitated again overnight with 50 μl of 10.5 M NH_4OAc and 500 μl of ethanol. After this precipitation, the samples were spun at 12,000 × *g* for 30 min at 4°C and vacuum desiccated, and the pelleted DNA was resuspended in 50 μl of Tris-EDTA (pH 7.8) at 37°C for 2 h and then stored at −80°C before use.

ARISA amplification. ARISA was conducted on 10 ng of extracted DNA as measured by Pico Green (Molecular Probes Inc.) fluorescence (9). The ITS regions (plus ~282 bases of 16S and 23S rRNA) of DNA extracts were amplified using PCR. PCR was carried out in 100-μl reaction mixtures using 1× PCR buffer, 2.5 mM $MgCl_2$, 250 μM (each) deoxynucleotide, 200 nM (each) universal primer 16S-1392F (5′-G[C/T]ACACACCGCCCGT-3′) and bacterial primer 23S-125R labeled with a 5′ TET (5′-GGGTT[C/G/T]CCCCATTC[A/G]G-3′), 5U of Taq polymerase (Promega), and bovine serum albumin (catalog no. 33036; 40-ng/μl final concentration; Sigma). These primers specifically targeted eubacteria; hence, archaea were not included in our analysis. Thermocycling was preceded by a 3-min heating step at 94°C, followed by 30 cycles of denaturing at 94°C for 40 s, annealing at 55°C for 40 s, and extension at 72°C for 90 s, with a final extension step of 5 min at 72°C. The calculated melting temperatures of both of the primers were ~52°C. The PCR amplification products were purified with Qiagen MinElute PCR purification kits and then diluted to 5 ng/μl as measured by Pico Green fluorescence. The products were then run in duplicate for 5 h on an ABI 377XL automated slab gel sequencer (2) with ABI FAM-labeled 2,500-bp standards. The sequencer electropherograms were then analyzed using ABI Genescan software.

Community analysis. Outputs from the ABI Genescan software were transferred to Microsoft Excel for subsequent analysis. Duplicate samples were analyzed for replicated peaks. Nonreplicated peaks (i.e., peaks which were present in one duplicate but absent in the second run of the same sample) and peaks <5 times the baseline fluorescence intensity were discarded. With these criteria, the practical detection limit for 1 OTU is ~0.09% of the total amplified DNA. The area under each peak was then averaged between replicates and expressed as a percentage of the total integrated area under the electropherogram (after first removing the areas of nonreplicated peaks). The Shannon-Wiener index (*H*) and Simpson's index (*D*), using descriptions given in reference 23, were determined according to the following equations:

$$H = -\sum_{i=1}^{n} P_i (\log_{10} P_i)$$

$$D = \sum_{i=1}^{n} (P_i)^2$$

where P_i is the fraction of the total integrated area in each peak. Due to concerns about artifacts associated with overamplification and small differences in the amounts of DNA run on the sequencer, these indices were calculated for all OTU that individually comprised >0.09% of the total amplified DNA, as well as only from OTU that individually comprised >0.5% of the total amplified DNA.

Pairwise similarities between whole communities (i.e., all OTU that individually comprised >0.09% of the total amplified DNA) were analyzed by manually calculating Jaccard coefficients (S_j) and Whittaker's index of association (S_w) (36) using the following equations (23):

$$S_w = 1 - \sum_{i=1}^{n} \frac{|(b_{i1} - b_{i2})|}{2}$$

$$S_j = W/(a_1 + a_2 - W)$$

where b_1 and b_2 are the percentage contributions to amplified DNA of the *i*th OTU in samples 1 and 2, respectively; *W* is the number of ITS peaks shared between populations 1 and 2; and a_1 and a_2 are the total numbers of different ITS lengths in populations 1 and 2, respectively. In identical communities, the differences between communities are 0, while in completely dissimilar communities, the differences between communities are 200% (i.e., 100% for each community). Thus, the sum of differences between communities is divided by 2 to generate the percentage dissimilarity. S_w is the complement of this, and as such, is the similarity between communities; for both Jaccard (presence-absence) and Whittaker (proportional) pairwise comparisons, the indices scale from 0 (completely different) to 1 (identical).

Correlation analyses between parameters were conducted using the statistical package in Microsoft Excel. Cluster analysis was conducted with the XLStat (AddinSoft SARL) program using the Jaccard coefficient or the Whittaker index of similarity and clustering analysis was conducted via the unweighted-pair-group mean-average method (32) after peaks were binned by size ± 1 bp for ITS lengths of <500 bp and ± 3 for ITS lengths of >500 bp (the accuracy of the fragment analyzer).

RESULTS

Bacterioplankton community ARISA. Bacterial-community fingerprints were different at each station sampled (Fig. 2), and a total of 118 taxa (phylotypes, or 1 ARISA length) each comprising >0.09% of the total amplified DNA were observed throughout the estuary. The estimated richness for this estuary system using Chao2 (5), which considers the number of taxa which occur only once across all sites and those that occur exactly twice, is 144 phylotypes. Diversity as measured by ARISA was variable throughout the estuary (Table 1). The greatest ARISA diversity and OTU richness occurred in the eastern portion of Moreton Bay at stations 4 and 6 (S = 84 and 85 OTU, respectively) while the least richness occurred at the most oligotrophic oceanic stations, 7 and 8 (S = 59 and 39 OTU, respectively). The richness within the Brisbane River estuary at stations 1 and 2 was similar to that at midbay stations (S = 60 to 77 OTU). The most abundant taxonomic unit (amplicon length, 662 bp) occurred in all samples and comprised 15.1 to 37.4% of the total amplified DNA. Several OTU were distinct to habitats, while some OTU appeared to be site

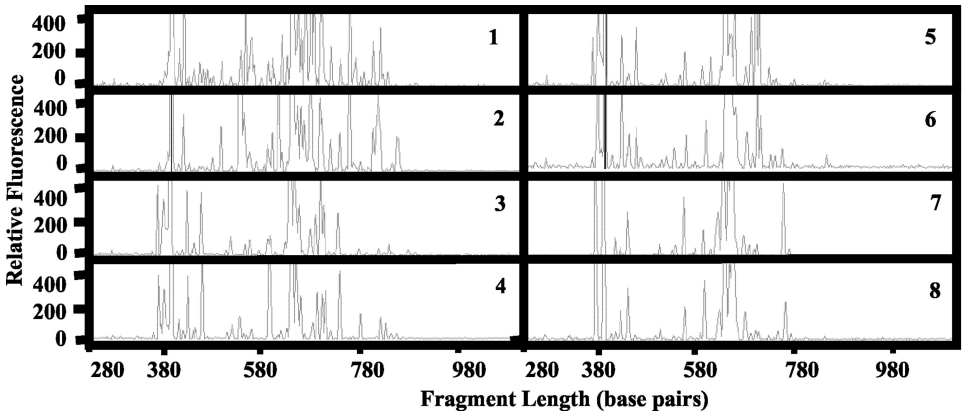

FIG. 2. ARISA electropherograms from Brisbane River-Moreton Bay estuary amplified 16S-23S ribosomal DNA amplicons. Station numbers are indicated in the top right corners of the electropherograms.

specific (Fig. 3). In total, 44 of 76 OTU, each comprising >0.5% of the total amplified DNA, were specific to a habitat type, while only 12 OTU displayed cosmopolitan distribution throughout the estuary. A further 14 OTU could be grouped more broadly into those occurring in pairs of adjacent habitat types (river and bay or bay and ocean).

Clustering and similarity analysis of bacterioplankton community fingerprints. As measured by ARISA, bacterioplankton communities were most similar between open-ocean sites (stations 7 and 8; Whittaker index = 0.71) and least similar among open-ocean, bay, and riverine sites (stations 2 and 5, Whittaker index = 0.38, and stations 2 and 7, Whittaker index = 0.39) (Fig. 4). There was moderate to strong similarity between communities within the Brisbane River (stations 1 and 2; Whittaker index = 0.65) and between communities in the bay portions of the estuary (stations 3 to 6; Whittaker index = 0.58 ± 0.01), indicating that there was relative homogeneity of the composition of bacterioplankton communities within environments but heterogeneity between environments.

Clustering analysis of bacterial-community fingerprints (Fig. 5) based upon the presence or absence of all observed taxonomic units (i.e., the Jaccard index) also demonstrated heterogeneity among riverine, bay, and open-ocean sites. Bacterioplankton communities in Moreton Bay (stations 3, 4, 5, and 6) were more similar to those at riverine stations (stations 1 and 2) than to oceanic sites (stations 7 and 8) when all observed OTU were used in the clustering analysis. A similar pattern, but with notably stronger clustering and clustering of bay stations with oceanic stations, was observed when clustering analysis was performed on only OTU comprising >0.5% of the total amplified DNA. This analysis demonstrated that there was also similarity between communities in the northern half of Moreton Bay (stations 5 and 6) and in the southern half of the bay (stations 3 and 4). Bacterioplankton diversity as measured by ARISA was not significantly correlated with salinity, nutrient (NO_3^- and PO_4^{3-}) concentration, chlorophyll *a*, bacterial abundance, or distance (Table 2); however, salinity was 35.5 PSU at all sites except in the river (5.5 to 28.3 practical salinity units [PSU]). There were, however, nonlinear correlations between bacterial-species richness and diversity (the Shannon-Wiener index) when OTU that were both >0.1% and >0.5% and bacterial abundance were considered (Fig. 6). A plot of the data shows a leveling off or even reduction of diversity at higher bacterial abundances (Fig. 6). The relationships between bacterial parameters and geographic distance from the river mouth suggested a maximum of both species richness and diversity in the bay portions of the estuary (a possible "hump-shaped" distribution). The temperatures at all stations were ±1°C and hence were not included in this analysis.

TABLE 1. Diversity statistics for all stations[a]

Station no.	Distance from river mouth (km)	*S*	$S_{0.5}$	*D*	*H*	$H_{0.5}$	Bacterial abundance (10^6 cells ml^{-1})
1	−27[b]	62	32	9.7	1.4	1.1	4.44 (±0.40)
2	0	77	32	9.9	1.3	1.1	4.35 (±0.44)
3	6	66	29	13.9	1.4	1.2	0.89 (±0.08)
4	27	85	33	17.5	1.5	1.2	1.74 (±0.13)
5	14	60	28	13.7	1.3	1.2	0.85 (±0.11)
6	43	84	29	6.2	1.3	1.0	0.64 (±0.11)
7	65	59	27	13.2	1.3	1.2	0.34 (±0.07)
8	80	39	26	9.0	1.2	1.1	0.30 (±0.09)

[a] *S*, OTU richness; *D*, Simpson's diversity index; *H*, Shannon-Wiener index. The subscript 0.5 indicates values calculated for ITS peaks that each comprised >0.5% of the total DNA. The values for *D* calculated only for peaks of >0.5% are not indicated because they were identical to those calculated for all taxa.
[b] Distance upriver from the river mouth.

Analysis of ARISA electropherogram data. For comparing the distribution of phylotypes within the bacterioplankton ARISA analyses, replicated peaks were divided into categories (>10, 5 to 10, 2 to 5, 1 to 2, 0.5 to 1, and 0.09 to 0.5% of the total amplified DNA) based upon each peak's contribution to the total amplified DNA. The main difference among the distributions of phylotypes in bacterioplankton populations was within phylotypes comprising <0.5% of the total amplified DNA (the "tail" of the species distribution curve) (Fig. 7), which ranged between 40 and 85 OTU, while phylotypes comprising 0.5 to 1% of the total amplified DNA had between 25 and 35 OTU. The number of phylotypes that individually comprised >0.5% of the community did not correlate with the amplicon amount, while the total number of peaks (i.e., all peaks with >0.09% of the total amplified

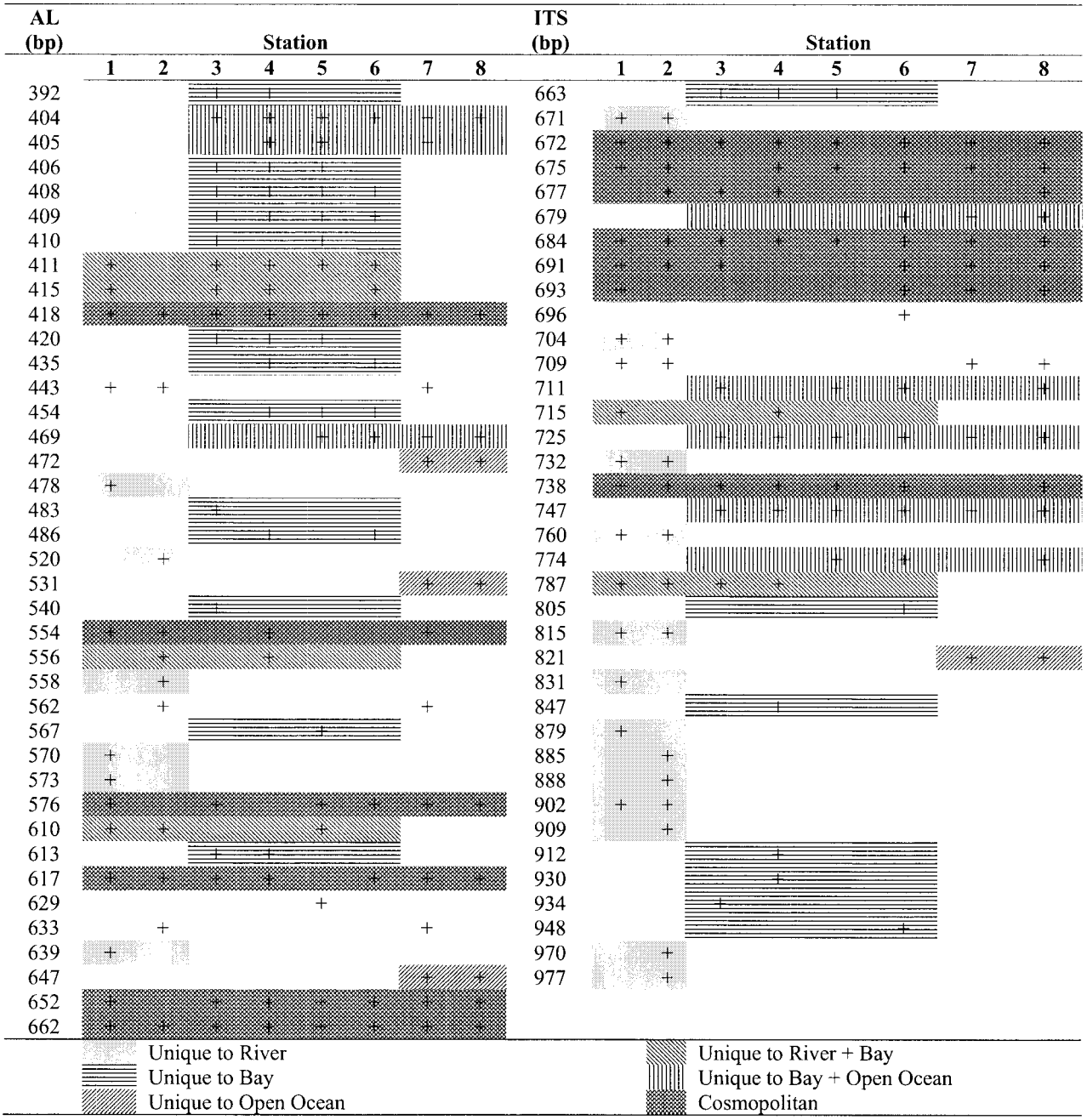

FIG. 3. ARISA OTU comprising >0.5% of total amplified DNA at each site. +, presence of a peak; AL, amplicon length.

DNA) did correlate (Fig. 8), indicating that differences in numbers of OTU may be an artifact of minor differences in the amount of amplicon analyzed. The Simpson's indices (which are less influenced by rarer taxa than the Shannon-Wiener indices) calculated only from phylotypes that each comprised >0.5% of the total amplified DNA were identical to those calculated from all phylotypes (Table 1). On the other hand, the Shannon-Wiener indices calculated only from phylotypes that each comprised >0.5% were different from those calculated for all OTU.

DISCUSSION

The results of this study indicate that amplified bacterioplankton communities within the Brisbane River-Moreton Bay estuary are diverse and that their composition changes from the riverine to open-water sites. However, these results also demonstrate that the diversity statistics at a single time are not linearly related to measured physical or chemical conditions but may change with habitat or location within the estuary. Since there is no linear correlation between instantaneous

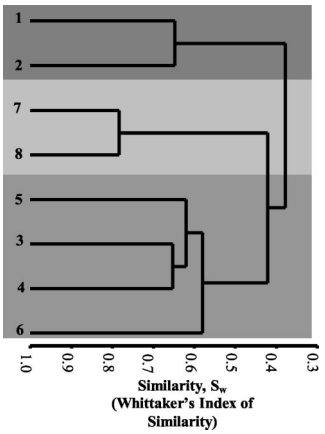

FIG. 4. Similarities, shown as Whittaker's index of similarity (23, 36), between bacterioplankton communities in the Brisbane River-Moreton Bay estuary considering all peaks of >0.09% and each OTU's relative contribution to the total amplified DNA. The average Whittaker's index between replicate analyses of the same sample is 0.89.

physical data and bacterial diversity in the Brisbane River-Moreton Bay estuary (Table 2 and Fig. 6), and only weak correlations between bacterial diversity (*H*) and chlorophyll concentration ($r = 0.15$), it is conceivable that diversity patterns may be driven by longer-term physical and chemical factors and productivity, which are integrated over several weeks or longer (33).

Amplified bacterial OTU richness as detected by ARISA changed along the estuarine gradient, with the highest amplified OTU richness in the eastern part of Moreton Bay, the lowest in the open ocean, and similar richness in the western part of Moreton Bay and within the Brisbane River. In contrast to OTU richness, diversity indices of bacterioplankton communities were highest at bay stations and in the East Australian Current but were lower in the Brisbane River, indicating a more even distribution in the East Australian Current. Previous studies of bacterial-species richness in estuarine environments have yielded similar results using both fingerprinting and cloning-and-sequencing approaches. Previous studies of bacterial diversity in a neritic environment (Yaquina Bay, Ore.) (34) and an estuary (Columbia River, Ore.) (6) using cloning and sequencing yielded observed and estimated richnesses of 60 and 269 species, respectively (ChaoI estimator [5]), while a study of bacterial diversity in the Rhone River and the Mediterranean Sea yielded ~85 phylotypes by using DGGE (35). This suggests that the number of OTU directly observed in Moreton Bay bacterioplankton (118 OTU) is within the range of richness reported in other coastal environments. However, clone library analyses probably offer more taxonomic resolution than fingerprinting analysis. The OTU richness observed in Moreton Bay is also higher than most other reports of aquatic DGGE OTU richness, which range from 6 to 85 phylotypes (for a review, see reference 35). Estimates of total OTU richness in this study, like all others to date, underestimate the total phylotype richness, since there are undoubtedly more rare taxa present in the tail of the species distribution curve that we cannot detect using this or any present protocol approach.

TABLE 2. Linear correlation (*r*) of bacterial diversity descriptive statistics and physical and chemical parameters at each station

Parameter	Correlation (*r*)							
	D	*H*	Salinity	NO_3	PO_4	Chlorophyll	Distance	Bacterial abundance
S	0.02	0.38	0.00	0.04	0.05	0.03	−0.11	0.08
D		0.58	0.08	−0.09	−0.08	0.02	−0.01	−0.02
H			0.02	0.02	0.02	0.15	−0.30	0.14
Salinity				−0.48	−0.37	−0.18	0.52	−0.72
NO_3					0.99	−0.02	−0.37	0.84
PO_4						−0.04	−0.32	0.79
Chlorophyll							−0.37	0.04
Distance								−0.63

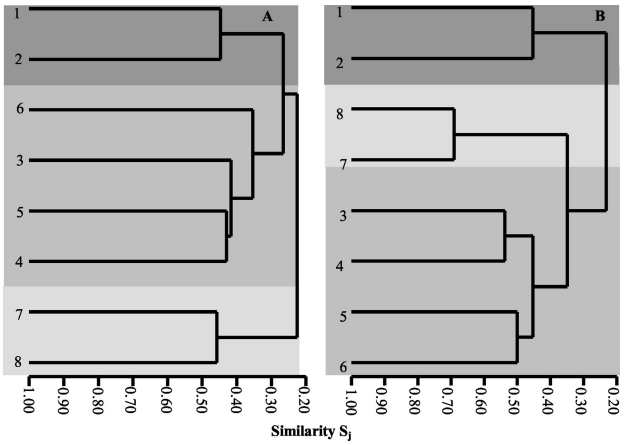

FIG. 5. Cluster analysis of bacterioplankton communities based upon all peaks observed (A) and only peaks comprising >0.5% of the total amplified DNA (B). Similarity is expressed as the Jaccard coefficient (S_j), which compares the presence or absence of OTU (not each OTU's relative contribution to the total amplified DNA) when making pairwise comparisons between communities, and clustering was performed with the unweighted-pair-group mean average.

Bacterial communities were different in the Brisbane River, Moreton Bay, and East Australian Shelf, as demonstrated by presence-absence (Fig. 5) or proportional clustering (Fig. 4) analysis. In addition, clustering analysis of major peaks (i.e., those comprising >0.5% of the total amplified DNA) demonstrated that bacterial communities in the northern half of the bay are different from those in the southern half. Interestingly, two out of the three major phylotypes (i.e., phylotypes comprising >5% of the total amplified DNA) were shared among all study sites; however, the OTU with an amplicon length of 686 bp is shared only among riverine sites, which is consistent with previous studies that have found that some dominant riverine bacteria are poor at surviving in marine waters (6, 35). Since relatively minor phylotypes (i.e., phylotypes that each comprised <5% of the total amplified DNA) could be either environment specific (i.e., riverine, bay, or open ocean) or common to all study sites, this suggests that the differences influencing the clustering analysis are not necessarily due to the presence or absence of minor phylotypes and include major phylotypes. The presence of a single phylotype (~662 bp) in all samples which comprise a large percentage of the total amplified DNA is intriguing. While no definitive identification of this

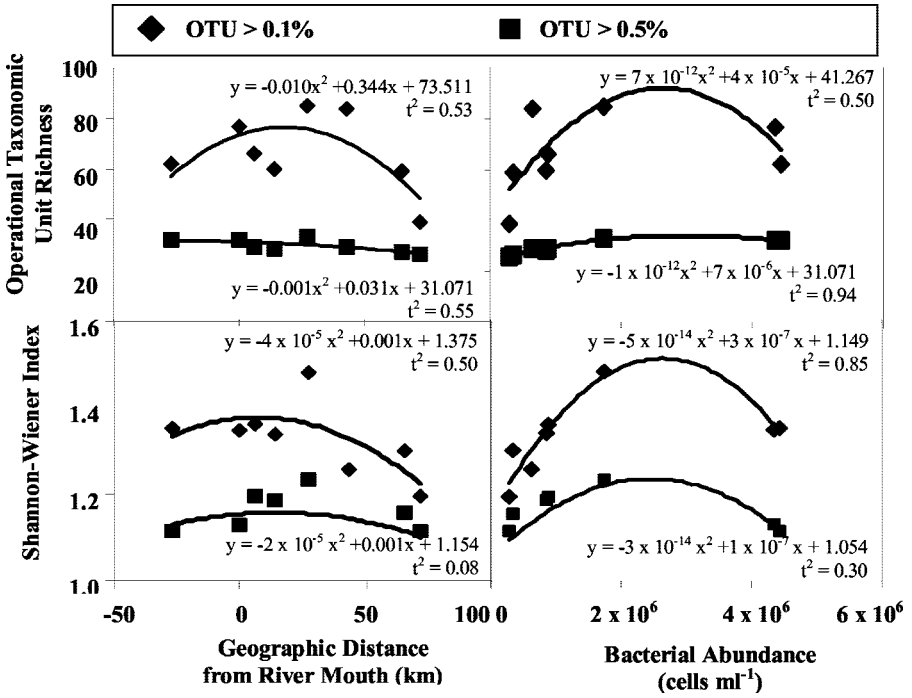

FIG. 6. Comparison of total OTU richness and diversity with geographic distance from the Brisbane River mouth and bacterial abundance.

peak is possible without a clone library from these samples, similar reports of ubiquitous bacteria exist in open-ocean studies (13, 24), and the 662-bp OTU and six other cosmopolitan OTU in this study are within the relatively narrow range of SAR 11 ITS lengths (660- to 711-bp fragment length, which includes ~282 bases of 16S and 23S ribosomal DNA in the ARISA PCR product) (12). This study demonstrates that a single bacterioplankton phylotype may be common from low-salinity (5.5-PSU) waters to the pelagic ocean in this estuary, which is consistent with a previous study of estuarine bacterial diversity in San Francisco Bay (16).

There are potential artifacts that need to be considered when using fingerprinting approaches for observing microbial diversity. Fingerprints may be influenced by variations in the amounts of DNA amplified, since more template can make otherwise undetectable rare peaks go above the detection limit. However, since a standard amount of DNA was used to conduct our PCR amplifications, our observations are not simply due to the amplification of a wider subset of bacteria more readily amplified from larger amounts of template from some samples. Additionally, the strong correlation observed between the amplicon amount and the number of OTU but lack of correlation between OTU that each comprised >0.5% of the total amplified DNA (Fig. 7) suggests that our inclusion of only these relatively abundant OTU (and not the tail of the species distribution curve) in one of the richness-abundance compar-

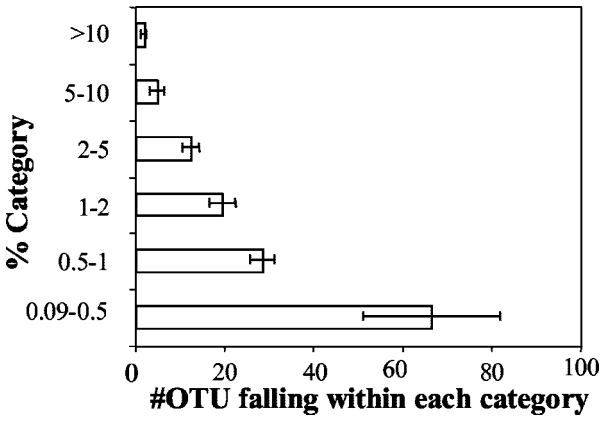

FIG. 7. Average (± standard deviation) number of OTU combined for all stations in categories of contribution to total amplified DNA.

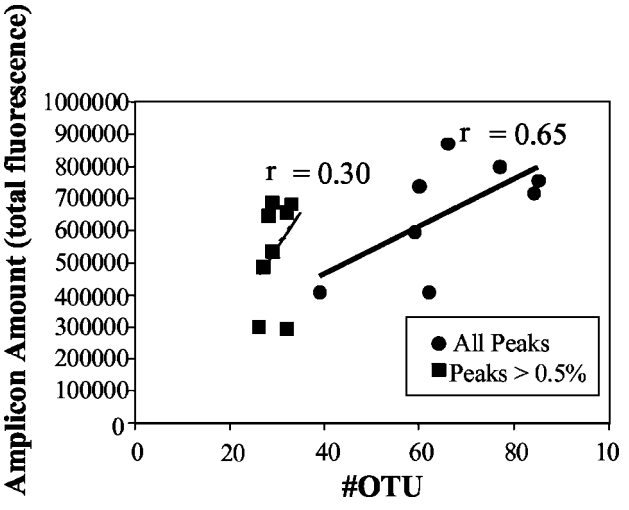

FIG. 8. Correlation between amplicon amount and total number of OTU, considering all peaks and only those which each comprised >0.5% of the total DNA.

isons (Fig. 6) minimizes artifacts associated with different DNA concentrations or minor changes in amplification efficiency. OTU richness calculated only from phylotypes that comprise >0.5% of the total amplified DNA yielded surprisingly similar values across all sites in this estuary (range = 27 to 33), whereas those that considered all phylotypes were heterogeneous (range = 39 to 85) (Fig. 7). The similar Simpson's indices calculated from phylotypes contributing both >0.5 and >0.09% of the total amplified DNA but different Shannon-Wiener indices calculated from these same criteria suggest that the main differences between bacterial dominances within sites is within the tail of the phylotype abundance curve (i.e., phylotypes comprising <0.5% of the total amplified DNA) (Table 1). Thus, in interpretation of ARISA data, care must be taken to avoid artifacts associated with OTU richness estimations from samples with different amplicon amounts.

It is important to note that calculated diversity indices in this study reflect diversity in ARISA amplifications and do not necessarily correspond exactly to bacterial-cell diversity in nature due to undetermined factors, such as variations in DNA per cell, operon copy numbers, or PCR bias. Along these lines, it is interesting that the relationships among stations (dendrograms) measured by presence-absence were substantially the same as those depending on quantitative information in Whittaker's index (Fig. 4 and 5B), so perhaps these biases are not large.

The distribution of total OTU richness in the bay (Table 1) demonstrates that a maximum of bacterial OTU richness and diversity occurs in the bay portions of the estuary, roughly following a bell-shaped distribution with respect to geographic distance from the Brisbane River mouth. Moreton Bay has a strong east-west trophic gradient, as described elsewhere (7, 15), ranging from highly productive waters (but low chlorophyll *a* due to light limitation) in the Brisbane River to low productivity in the East Australian Current. Contrasting relationships have been observed between bacterial diversity and productivity in other studies. Recent observations of aquatic diversity and richness show maximum diversity at intermediate productivity (31). A study using the bacterium *Pseudomonas fluorescens*, which undergoes very rapid genotypic variation specialized to niches, indicated that diversity increased monotonically with productivity in homogeneous (i.e., unshaken) incubations, while diversity was maximal at intermediate productivity in a heterogeneous (i.e., shaken) environment (20). Our data are consistent with this culture study, since the Moreton Bay estuary is a heterogeneous environment and maximum bacterial richness was observed at intermediate productivity (7), while more productive waters (e.g., upriver in the Brisbane River [7]) did not yield richer communities. In contrast to reports of linear increases in diversity with elevated productivity, several studies have demonstrated decreased diversity of marine organisms with increased productivity (e.g., diatoms in polluted streams [29] and benthic invertebrates near a sewage outfall [1]). To a large extent, this effect is not due to low richness but rather to stronger dominance by a few species. The decreased diversity of abundant riverine bacterioplankton observed in this study may be due to elevated nutrient concentrations, which may support the growth of faster-growing species that may outcompete slower-growing species. Interestingly, a study of bacterial OTU richness along a freshwater productivity gradient in mesocosms demonstrated that while there was no overall pattern between diversity of bacterial communities and productivity, there were both humped and U-shaped distributions between the richness of bacterial taxonomic groups (e.g., α- and β-proteobacteria) and productivity as measured by chlorophyll *a* concentrations (17). Our methods preclude the calculation of within-group diversity, since we have not identified OTU.

Study of estuarine bacterial composition in the Columbia River using a cloning and sequencing approach demonstrated that ~48% of clones in estuarine bacterioplankton were in common between ocean and riverine communities (6). This mixing of bacterial communities in intermediate waters may also occur in the bay portions of the Moreton Bay estuary, where greater species richness was observed. Since the residence time of seawater at bay stations is short (6 to 48 days) (7), there is probably insufficient time for ecological factors, such as niche differentiation in bacterioplankton communities, to lead to an equilibrium situation where competitive exclusion may occur.

Previous studies of coastal bacterioplankton communities with DGGE and TRFLP have shown homogeneous bacterioplankton communities across different water masses and across time (30). For example, study of the Rhone River plume using DGGE indicated similar numbers of phylotypes in riverine, plume, and open-ocean sections of the estuary, and there was no correlation between OTU richness and salinity or nutrient concentrations or between OTU richness and bacterial activity (35). This study contrasts with previous results by suggesting that bacterial communities are heterogeneous spatially over trophic gradients, even within a relatively small area. While our study demonstrates that there can be common phylotypes which comprise a large percentage of bacterioplankton communities, our results also contrast with the previous study in that rarer phylotypes are different across different estuarine water masses.

It is important to note that samples were prefiltered through 0.7-μm-pore-size filters to exclude protists and plastid DNA from our analyses, which would have made it extremely difficult to interpret fingerprints at all, since the universal primers used in this study would have also amplified these components. However, this prefiltration may have contributed to selective underestimation of bacterial community diversity and OTU richness, since bacteria >0.7 μm in diameter are common in bacterioplankton communities. Additionally, this prefiltration would also exclude particle-attached bacteria, which also probably occur in high suspended-solid portions of the estuary (e.g., in the Brisbane River). Hence, our results apply to the free-living bacteria of <0.7 μm, estimated at about half of the total abundance (22). Nevertheless, this study also demonstrates the usefulness of a fingerprinting approach in observing bacterioplankton community patterns.

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